REMARKS

This is in response to the Office Action mailed October 1, 2010. Claims 1-10, 12, 13, 15-17, and 21-22 are pending. Claims 18-20 are withdrawn. With entry of this amendment, claims 1-10, 12, 13, and 15-22 are active. No new matter is added with this amendment.

I. Claim rejections – 35 USC § 112

The Examiner rejects claims 2-3 under 35 USC § 112, second paragraph, as being indefinite for reciting "about" in claim 2. In response, applicants herewith delete "about" in claim 2. Withdrawal of this rejection is therefore respectfully requested.

II. Claim rejections – 35 USC § 103

Claims 1, 4-6, 15 and 17 remain rejected under 35 USC 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") in view of Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle"). Applicants traverse this rejection.

According to the Examiner, applicants' prior arguments to rebut this rejection are unpersuasive because the claims are not interpreted as excluding the detection of visible microcolonies caused by the fluorescent part of the fluorogenic substrate released from each single microorganism (Office Action at page 9). The Examiner says that "the instant claims do not specify that background fluorescence is not registered in order to improve sensitivity and specificity and thus is not commensurate with the scope of the claims" (Office Action at page 9, third full paragraph).

In response, applicants have amended claims 1 and 21 to recite "A method for detecting and counting individual intracellularly labeled microorganisms in a sample, wherein background fluorescence is avoided." Part d) of claims 1 and 21 are similarly amended to clarify that because the fluorescent labels are retained in the microorganisms, background fluorescence is avoided. Part e) is amended to clarify that each microorganism is detected and counted. Support for this amendment can be

found in a combination of paragraph [0030], which discusses the prior art problem of background noise, and paragraphs [0096] and [0098], which prescribe retaining the fluorescence inside the cells and using labels based on their capacity to remain inside the cells. Paragraph numbers correspond to the numbers in the present application as published (Application No. 20050202519). Applicants have previously explained that the retention of the fluorescence in the cell results in a faster and more sensitive assay. This amendment renders the claims to be commensurate in scope with these previous arguments. In view of this amendment, applicants respectfully request the Examiner to reconsider and withdraw the rejection of all the claims over a combination of Berg and Pyle with or without further secondary references.

Applicants also again emphasize that Berg teaches detecting colonies; Berg does not teach detecting individual microorganisms. This distinction is relevant because, unlike the present invention, Berg's method does not permit the counting of bacteria that have lost their ability to multiply. The ability to count bacteria that have lost their ability to multiply is an advantage achieved by the present invention in that it provides a more accurate count (paragraphs [0064] and [0065]). Also, as applicants have previously pointed out, because the claimed method does not depend upon the growth of a microcolony, it is a faster process. For instance, the growth of a colony from an agar medium may take from 24 to 48 hours. In contrast, the detection of intracellular fluoresecene emitted by an individual microorganism and detected by a method in step e) takes only a few minutes (paragraph [0062]). Applicants have amended claims 1 and 21, part e) to clarify that each individual microorganism is detected and counted so that the scope of the claims is commensurate in scope with the above arguments that distiniguish the claimed invention over the teachings of Berg.

Applicants also want to clarify what Berg teaches with regard to "detecting and counting." Berg discloses two different embodiments. In the first embodiment at pages 6 and 7, only the amount of emitted fluorescent light is detected (page 7, lines 1 and 2). The second embodiment, which utilizes an agar medium, is described at page 8, second paragraph, to page 9, third paragraph. The second embodiment involves counting the

number of fluorescent microcolonies, but not individual intracellularly labeled cells, in step 4). Similarly, in claim 1, Berg does not teach counting individual cells but rather the step of measuring the amount of the emitted fluorescence to calculate the velocity of emitted fluorescence.

Applicants also want to address the Examiner's comments regarding the use of a permeability enhancer in Berg. Applicants maintain that background fluorescence is not avoided by the method of Berg and that the use of a permeability enhancer in Berg supports this assertion. In Example 1 of Berg, it appears that the permeability enhancer permits an increase in the release of fluorescence and that one of skill in the art reading Berg would be motivated to use a permeability enhancer for the purpose of improving the release of extra cellular fluorescence. Applicants' amendment clarifies that the method of the claimed invention can be distinguished over Berg in this regard.

The Examiner has cited Pyle for teaching the step of immunomagnetically concentrating the microorganisms that are detected and counted. Applicants argue that one of skill in the art could not combine the teachings of Pyle with those of Berg and arrive at the claimed invention. Pyle does not remedy the deficiencies in Berg, as discussed above. That is, like Berg, Pyle does not teach a step of detecting and counting individual intracellularly fluorescene-labeled microorganisms. In addition, Pyle does not teach detecting and counting such labeled cells by way of flow cytometry, filtration cytometry and fluorescene microscopy, as required by the claim 1. Furthermore, Pyle does not disclose or suggest: a step of selectively enriching the microorganism sought in the sample; the use of a substrate comprising one part specific to the enzymatic activity; or the induction/activation of at least enzymatic activity of the microorganism. Accordingly, one of skill in the art reading Pyle would not have been able to combine its teachings with those of Berg to arrive at the claimed invention. Withdrawal of the rejection of claims 1, 4-6, 15 and 17 for obviousness over Berg and Pyle is respectfully requested.

Claim 16 remains rejected under 35 USC § 103(a) as being unpatentable over

Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") and Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle") as applied to claims 1, 4-6, 15 and 17 above, and further in view of Sigma catalog 1996 p. 2179-2181. Applicants respectfully traverse this rejection. The Examiner cites Sigma catalog for teaching filters with different pore sizes. Claim 16 depends indirectly from claim 1. Applicants maintain that Sigma catalog does not cure the deficiencies of Berg alone or when combined with Pyle for reasons set forth above. Accordingly, applicants respectfully request the Examiner to withdraw this rejection.

Claims 8-10 remain rejected under 35 USC § 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") and Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle") as applied to claims 1, 4-6, 15 and 17 above, and further in view of Olsen *et al.* (Plant and Soil 186:75-79, 1996, "Olsen"). Applicants respectfully traverse this rejection. Claims 8-10 depend directly or indirectly from claim 1. The Examiner cites Olsen for teaching immunomagnetic concentration guidelines. Applicants maintain that Olsen does not cure the deficiencies of Berg alone or in combination with Pyle for reasons set forth above. Accordingly, applicants respectfully request the Examiner to withdraw this rejection.

Claims 12 and 13 remain rejected under 35 USC § 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") and Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle") as applied to claims 1, 4-6, 15 and 17 above, and further in view of Boyd *et al.* (US 5,510,243, April 23, 1996, "Boyd"). Applicants respectfully traverse this rejection. Claims 12 and 13 depend directly or indirectly from claim 1. Boyd is cited for teaching a fluorogenic label that is a xanthene. Applicants maintain that Boyd does not cure the deficiencies of Berg alone or in combination with Pyle for reasons set forth above. Accordingly, applicants respectfully request the Examiner to withdraw this rejection.

Claim 7 remains rejected under 35 USC § 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") and Pyle *et al.* (WO 95/31481 23

November 1995 "Pyle") as applied to claims 1, 4-6, 15 and 17 above, and further in view of Kaclikova *et al.* (Journal of Microbiological Methods, Vol. 46, Issue 1, July 2001, p. 63-67, "Kaclikova"). Applicants respectfully traverse this rejection. Claim 7 depends from claim 1. The Examiner cites Kaclikova for teaching the use of Fraser broth as enrichment media in a method of detecting *Listeria*. Fraser broth is said to comprise yeast. Applicants maintain that Kaclikova does not cure the deficiencies of Berg alone or in combination with Pyle for reasons set forth above. Accordingly, applicants respectfully request the Examiner to withdraw this rejection.

Claims 2-3 and 21-22 remain rejected under 35 USC § 103(a) as being unpatentable over Berg *et al.* (WO 89/04372, 18th May 1989, "Berg") and Pyle *et al.* (WO 95/31481 23 November 1995 "Pyle") as applied to claims 1, 4-6, 15 and 17 above, and further in view of Strenkoski *et al.* (US 5,843,699, Dec. 1, 1998 "Strenkoski"), and Heck *et al.* (US 3,704,204, Nov. 28, 1972, "Heck") and Ray, Bibek (Injured Index and Pathogenic Bacteria, 1989, CRC Press Inc., Boca Raton, Florida, P. 78, "Ray") and Patel *et al.* (Journal of Food Protection, 1995, Vol. 58, No. 3, p. 244-250, "Patel"). Claims 2-3 depend directly or indirectly from claim 1.

The Examiner acknowledges that the combination of Berg and Pyle do not teach enriching microorganisms in sodium pyruvate, sodium thiosulfate and catalase. The secondary references are said to provide such teachings. Applicants maintain that none of Strenkoski, Heck, Ray or Patel, alone or in combination, cure the deficiencies of Berg alone or in combination with Pyle for reasons set forth above. Accordingly, applicants respectfully request the Examiner to withdraw this rejection.

In view of the above explanations, applicants respectfully request the Examiner to reconsider and withdraw all of the rejections for obviousness.

CONCLUSION

In view of the above amendment and explanations, applicants believe all rejections under 35 USC § 112 and § 103 should be withdrawn. A Notice of Allowance is respectfully requested. Should the Examiner believe that anything further is necessary in order to place this application in better condition for allowance, the Examiner is requested to contact the undersigned at the telephone number listed below.

In the event that an extension of time is necessary to prevent abandonment of this application, then such extension of time is hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account No. 01-2300 referencing docket number 029440.00009.

Respectfully submitted,

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